Winemaking by-products as a sustainable source of antioxidant and functional compounds

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Abstract. In the last years, the international guidelines encourage the reduction of food waste or processing by-products to promote a circular economy approach. Winemaking represents one of the sectors with the highest by-products generation, with several potential negative impact on the environment. Winemaking by-products are mainly used to produce distillates or fertilizers, but the interest in grape pomaceas a potential source of phenolic compounds has considerably increased. The aim of this study was the application of *in vitro* methods for the characterization of the phenolic fraction of winemaking by-products from different red grape varieties, and, in parallel, the evaluation of their antioxidant activity. The methods were: 1) Folin-Cocalteau's assay for the quantification of total polyphenol content; 2) the vanillin assay for the assessment of total flavan-3-ols (proanthocyanidins) content; 3) the pH differential method for the quantification of total anthocyanin content; 4) DPPH assay for the measurement of total antioxidant activity; 5) High Performance Thin Layer Chromatography for separation of phenolic substances and assessment of their antioxidant activity. Although a significant differences among varieties, grape pomace, particularly when containing seeds, are generally a good source of polyphenols with a significant antioxidant activity supporting itsuse in formulating healthy products.

1 Introduction

Grape (*Vitisvinifera* L.) is one of the most widespread crops in the world, with approximately 75 million tons produced every year [1]. In 2021, the global vineyard area was estimated to be 7.3 mha, and more than 50% of grapes were used for wine production, which was estimated at 260 mhL [2].According to the last OIV statistical report on wine vitiviniculture (2021), about 56% of the total wine is produced in Europe, followed by South America (11.4%) and USA (9.3%). The winemaking generates high amount of secondary products with a negative impact on the environment, and the production of organic and solid grape wastes represents a pressing environmental issue in European wine production.

For this reason, both the European Green Deal and the OIV (strategic plan 2020-2024), encourages circular economy through the reuse of waste and management of by-products, defining and developing guidelines on "green" chemistry.

The main winery by-products (Fig. 1) are the grape marc or pomace, which represents between 20 and 25% of the initial grapes' weight.

It is composed of 25% seeds, 25% stalks, and 50% skins (left after the crushing and pressing stages of wine production) [3]. One of the most important destination of the pomace is distillation [4], and, to a less extent, it can be used as a fertilizer or a microbial substrate for acid lactic production. Grape stalks contain high levels of cations (K and Fe mainly) that can be used for soil amendment [5].

Grape seeds are generally used to produce oil and meal for human and animal consumption, respectively. The seeds and the skins are also rich in soluble fibers, unsaturated lipids, sterols, vitamins, polyphenols and other antioxidants [6]. The content of phenolic compounds, representing about 2-3% of the grape pomace, has raised a great interest since these moleculeshave been associated with the reduction of risk factors for several diseases [6].

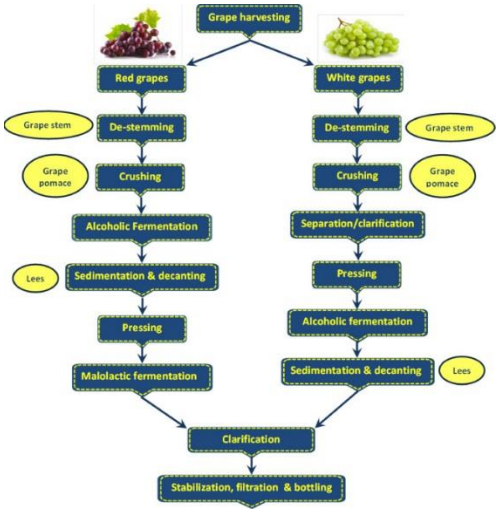


Figure 1. Winemaking steps for red and wine production and main by-products generated. Adapted from Chakka et al. 2022 [7].

The main phenolic components of grape marc are flavonoids, and in particular flavanols, flavonols and anthocyanins. Grape skins of pomace from red varieties contain mainly anthocyanins. The most important anthocyanins present in red skins are the 3-O-monoglucoside and the 3-O-acylated monoglucosides of: delphinidin, cyanidin, petunidin, peonidin and malvidin. Grape seeds, skin and stems are also an important source of proanthocyanidins (PROs), oligomers and polymers of

(+)-catechin, (-)-epicatechin, and (-)-epicatechingallate. Skin and stems also containprodelphinidins, oligomers and polymers of (-)-epigallocatechin and trace amounts of (+)-gallocatechin and (-)-epigallocatechingallate [8]. PROs and non-flavonoid compounds are considered responsible for several healthy effects; among them, the improvement of the endothelial function, the increase of the serum antioxidant capacity, the protection of LDLs against oxidation and the reduction of inflammation [9, 10]. For these reasons, the recovery of these compounds after the winemaking could be an interesting process to reduce the ecological impact of vinification and, in parallel, to formulate food supplements or enriched foods.

Table 1. Samples included in the study, year of collection, winemaking stage of collection and codes used in the paper.

Samples		**Winemaking stage**	**CODE**
Grignolino, 2015	Seeds	Initial fermentation (2° day)	G-IF-S-15
Grignolino, 2015	Seeds	After fermentation	G-AF-S-15
Grignolino, 2016	Seeds	Initial fermentation (2° day)	G-IF-S-16
Grignolino, 2016	Seeds + skins	After fermentation	G-AF-SS-16
Grignolino, 2018	Seeds + skins	After fermentation	G-AF-SS-18
Uvalino, 2015	Seeds	Initial fermentation (2° day)	U-IF-S-15
Uvalino, 2015	Seeds + skins	After fermentation	U-AF-SS-15
Uvalino, 2016	Seeds + skins	After fermentation	U-AF-SS-16
Barbera, 2017	Seeds + skins	After fermentation	B-AF-SS-17

On this basis, the aim of the study was a preliminary characterization of the phenolic pattern and the measure of antioxidant activity of different winemaking by-products collected from different red *Vitisvinifera* cultivar.

2 Materials and methods

The methods developed for the characterization of the phenolic fraction of pomace samples were based both on spectrophotometric and chromatographic approaches. Spectrophotometric methods included: 1) Folin-Ciocalteau's assay for the quantification of total polyphenol content; 2) the pH differential method for the quantification of total anthocyanin content; 3) vanillin assay for the determination of total flavan-3-ols (proanthocyanidins); 4) DPPH assay for antioxidant activity evaluation. HPTLC (High Performance Thin Layer Chromatography) was used as fast chromatographic approach for the separation and semi-quantitative evaluation of antioxidant property of pomaceactive compounds.

2.1 Samples

The samples included in the study, kindly provided by Dr.AntonellaBosso, CREA (Asti, Italy) were composed by different winery by-products from red varieties collected in different winemaking stages (Table 1).

All samples were maintained at -20°C till the use.

2.2 Spectrophotometric assays

Four spectrophotometric assays were used in this study.

2.2.1 Folin-Ciocalteu's assay

Total polyphenol content (TPC) was determined according to Singleton and Rossi [11].

About 0.4 g of each blended sample were mixed with 3 mL methanol:water (1:1) mixture, sonicated for 15 minutes using an ultrasonic bath and centrifuged for 15 minutes at 8000 r.c.f. (relative centrifugal force) at 4°C. The supernatant was collected and filtered on a paper filter. A second extraction was performed on the solid precipitate; the two supernatants were combined and adjusted to volume (5 mL) with methanol:water (1:1) mixture. Aliquots of 300 µL from samples, or water for blank, were mixed in test tubes with: 1.5 mL of Folin-Ciocalteau's reagent (Sigma Aldrich, Germany) diluted 10 times, and 1.2 mL of 7.5% sodium carbonate (Sigma Aldrich, Germany). After 30 minutes, the absorbance was measured at 765 nm in a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, California, U.S.A.). Each sample was extracted in triplicate. Results were expressed as mg/g gallic acid (GA) equivalents (dry weight).

2.2.2 Total Anthocyanin Content

Total anthocyanin content of red pomace samples was determined according to the AOAC method [12]. About 0.4 g of each blended sample were mixed with 3 mL of methanol:HCl 85:15 (v/v), sonicated for 15 minutes using an ultrasonic bath and centrifuged for 15 minutes at 8000 r.c.f. (relative centrifugal force) at 4°C. A second extraction was performed on the solid precipitate; the two supernatants were combined and adjusted to volume

(5 mL) with methanol:HCl 85:15 (v/v). The absorbance of samples, prepared as described in 2.2.1 and suitably diluted with pH 1.0 (0.025M potassium chloride) and pH 4.5 (0.4M sodium acetate) buffers, were measured both at 520 and 700 nm, using the last lecture to correct for haze. Each analysis was performed in triplicate. Results are expressed according to the following relationship (1) where antocyanin pigments (AP) are expressed as cyd-3-glu equivalents (mg/L):

$$AP\ (mg/L) = A \times MW \times DF \times 1000/e \times 1 \quad (1)$$

where: $A = (A_{520nm}-A_{700nm})_{pH\ 1.0} - (A_{520nm}-A_{700nm})_{pH\ 4.5}$; MW (molecular weight) = 449.2 g/mol for cyd-3-glu; DF = dilution factor; l = path length in cm; e (molar extinction coefficient) = 26,900 for cyd-3-glu; 1000 is the factor for conversion from g to mg.

2.2.3 Vanillin assay

The totalcontent of monomeric and condensed flavanols (proanthocyanidins) was measured by vanillin assay [13]. The vanillin reaction involves an aromatic aldehyde, vanillin, that reacts with meta-substituted ring of flavanols to yield a red adduct, with a maximum absorbance at 500 nm. About 0.5 g of blended samples were extracted with 10 mL of methanol and stirred with a magnetic stir for 20 min in the dark. Then, the solutions were centrifuged for 10 minutes at 8000 r.c.f. at 4°C and filtered with a paper filter. The supernatants were then collected and opportunely diluted. The extraction procedure was performed in triplicate. Catechin standard solutions were prepared in methanol using catechin in the range of 50-200 µg/mL. Vanillin reagent was prepared using 1% methanolic solution of vanillin (1%, w/v) mixed with 3% HClmethanolic solution (v/v). Aliquots of 0.5 mL of samples or standard solutions were added with 2.5 mL of vanillin reagent (VR) or 1.5% HCland maintained at 30°C form 20 minutes in the dark. Then, the absorbance was measured spectrophotometrically at 500 nm. The Δ absorbance was calculated as follows:

$$\Delta\ absorbance: (A\ sample\ VR - A\ blank\ VR) - (A\ sample\ HCl - A\ blank\ HCl) \quad (2)$$

A standard curve was obtained by correlating absorbance values with catechin concentrations. Results wereexpressed as mg catechin (C) equivalents/gof grape by-product.

2.2.4 Antioxidantactivity by DPPH assay

The antioxidant activity (AOA) of grape pomace was measured spectrophotometrically, as a measure of radical scavenging activity, using 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) [14, 15]. Samples were prepared as described in 2.2.1. Standard solutions of gallic acid (GA) were prepared in methanol:water 1:1 (v/v) in the range of 1-5 µg/mL.Aliquots of 1 mL of DPPH (Sigma Aldrich, Germany) in methanol (5 mg/100 mL) were mixed with 0.5 mL of standard solution or sample suitably diluted. The absorbance was measured after 30 minutes at 517 nm. Results were expressed as equivalents of gallic acid (GA) in mg/g of sample.

2.3 Fast chromatographic methods: High Performance Thin Layer Chromatography (HPTLC)

Thin Layer Chromatography (HPTLC) is a fast and suitable method for screening different classes of molecules, allowing the fingerprint characterization of several botanical products [16]. Furthermore, HPTLC technique can be used for the assessment of biological properties such as, for example, the semi-quantitative measure of antioxidant activity.

In this study, HPTLC technique was used to perform a screening of the most representative polyphenol classes (phenolic acids, flavonols and flavanols) in grape by-productsand evaluate in parallel the associated antioxidant activity.

2.3.1 Polyphenol profile and antioxidant activity of pomace samples

Aliquots of 10 µL of standard solutions (200 µg/mL) of the main pomacepolyphenols (kaemferol-3-glu, hyperoside, caftaric acid, quercetin-3-O-glu, epicatechin) were applied on silica-gel plates. Sample volumes of 5 µL samples, prepared as described in 2.2.1, were loaded onto the plate. At the end of the chromatographic run, performed using 10 mL of mobile phase (toluene:acetone:formic acid 4.5:4.5:1) the plate was sprayed with a DPPH (Sigma Aldrich, Germany) methanolic solution (0.05%) and dried for 1 min at room temperature in an extractor hood. The dried plate was wrapped with aluminium foil for 30 min and exposedat UV (366 nm) or at visible light.

3 Results and Discussion

3.1 Spectrophotometric assays

Table 2 reports total polyphenol content (TPC), total proanthocyanidin content (Tpro) and the antioxidant activity (AOA) of the samples analyzed.

Table 2. Mean concentrations (± SD; *n*=3) of TPC, TPro and AOA in the samples analyzed.

Sample	TPC mg GA/g	TPro mg C/g	AOA mg GA/g
G-IF-S-15	42.85±2.60	29.16±1.41	34.50±1.38
G-AF-S-15	26.46±3.27	11.51±0.81	26.32±0.51
G-IF-S-16	24.00±0.62	24.86±1.80	17.73±2.05
G-PF-SS-16	9.00±0.43	6.70±0.49	5.19±0.23
G-PF-SS-18	9.74±0.12	4.49±0.44	3.54±0.37

U-IF-S-15	50.82±0.90	42.34±2.46	42.2±3.37
U-AF-SS-15	12.36±1.44	6.23±0.64	7.48±0.25
U-AF-SS-16	8.47±0.58	2.08±0.06	4.05±0.17
B-AF-SS-17	9.60±0.38	2.66±0.19	5.19±0.31

Total phenolic content (TPC) and total proanthocyanidins (TPro) of Grignolino seeds collected in 2015 was reduced by about 50% after the winemaking. The significant amount of proanthocyanidins found after the fermentation can be due to the high concentration of these compounds in the seeds, where can reach 52% (w/w) [17]. Total antioxidant activity (AOA) was less affected by winemaking, since a decrease by about 24% was observed.

This could be due to the high radical scavenger capacity of proanthocyanidins and to the contribution of other antioxidant molecules such as phenolic acids (caftaric acid, chlorogenic acid, and caffeic acid) [18]. In the seeds collected from Grignolino2016, the initial TPC (42.85±2.60 mg GA/g) was about half than the content found in the seeds in 2015 (24.00±0.62 mg GA/g), meaning that different factors, such as climatic conditions and degrees of ripeness could have affectedthe quali-quantitative polyphenol composition in samples collected in different years. On the other hand, total proanthocyanidin content in Grignolino sample (2016) containing only seeds was comparable to that found in the 2015 (only seeds) supporting our hypothesis that other phenol compounds contributed to TPC in 2015 [19]. In 2016, after fermentation, a strong decrease of TPC, TPro and AOA was observed in Grignolino samples, due to the additional presence of the skins that represent until 50% (w/w) of grape pomace. Furthermore, polyphenols in grape skins, composed mainly by anthocyanins, are generally highly affected by winemaking due to their higher water-ethanolic solubility and the greater surface area exposed to the fermentation process. Similar observations can be done for Grignolino by-products collected in 2018. Uvalino seeds (2015) presented a TPC similar to Grignolino, but the proanthocyanidin content was significantly higher. Similar considerations can be done for Uvalino by-products collected in 2015 and 2016 and for Barbera (2017). The correlation between TPC, TPro (flavan-3-ols) and AOA (Table 3) was evaluated using Pearson's correlation coefficients (threshold for statistical significance: $p<0.01$). The statistical analyses were carried out with IBM SPSS Statistics, Version 27.0.

Table 3. Correlation between parameters measured with spectrophotometric assays in the by-product samples (n = 9 samples).

Parameter	Pearson correlation coefficient (r)	statistical significance (p)	Strength
Total polyphenols vs Total flavan-3-ols	$r = 0.946$	$P<0.001$	Positive strong correlation
Total polyphenols vs Antioxidant capacity	$r = 0.983$	$P<0.001$	Positive strong correlation
Total flavan-3-ols vs Antioxidant capacity	$r = 0.909$	$P<0.001$	Positive strong correlation

Both TPC and TPro were significantly well correlated with AOA, suggesting that, among polyphenols, flavan-3-ols highly contribute to the total antioxidant capacity of samples. In Figures 2 and 3 were illustrated the linear regression between TPC and AOA and TPro and AOA, respectively.

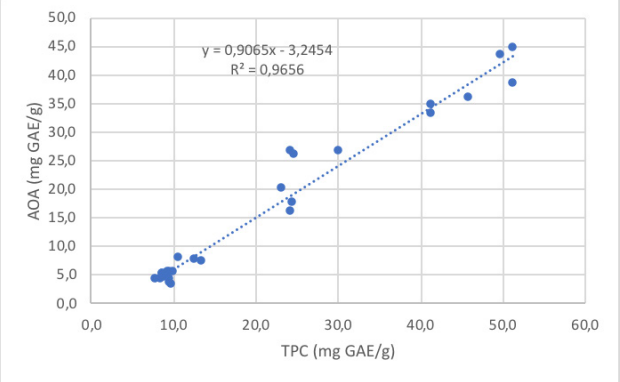


Figure 2. Linear regression between total polyphenol content (TPC) and antioxidant activity (AOA). Results are expressed as mg of gallic acid equivalent (GA)/g.

Compared to TPC vs AOA, aworse linear correlation between proanthocyanidins and AOA (R^2=0.82) (Fig. 3) was observed, suggesting that compounds different from proanthocyanidins could contribute to AOA, for example anthocyanins.

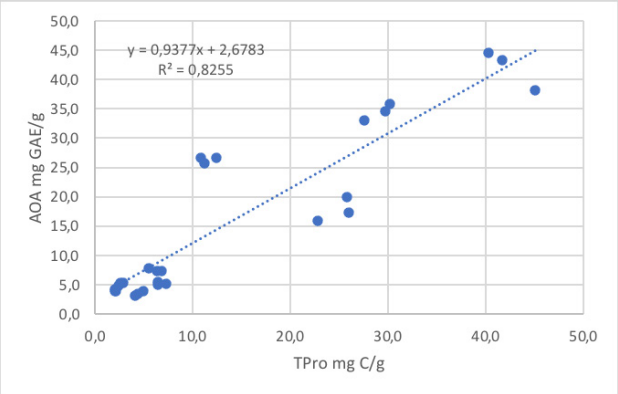


Figure 3. Linear regression between total proanthocyanidins (TPro) and antioxidant activity (AOA). Results are expressed as mg of catechin equivalent (C)/g for TPro and mg of gallic acid equivalent (GA)/g for AOA.

In Figure 4, total anthocyanin content (TAC) of samples are reported. Data were subjected to one-way analysis of variance (ANOVA), followed by the Duncan test to identify significant differences.

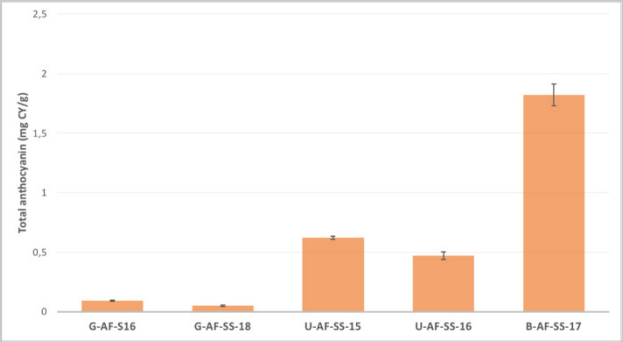


Figure 4. Total anthocyanin content (TAC) (mg/g of cyanidin equivalents±SD; *n*=3) of winemaking by-products containing skins. Data having different letters are significantly different (p<0.001). For abbreviations, see Table 1.

Total anthocyanin content ranged between 0.05±0.007 mg/g (Grignolino 2018, G-AF-SS-18) and 1.82±0.091 mg/g (Barbera 2017, B-AF-SS-17). After fermentation, no significant differences were found between TAC of Grignolino samples collected in different years, while TAC of Uvalino 2015 showed a little, but significant difference respect to the same variety collected in 2016. This observation suggests that similar differences were present in the berries before the fermentation. Factors influencing the anthocyanin content in grapes belonging to the same cultivar over different years generally include the evolution of climatic characteristics (rainfall, temperature, and relative humidity). Furthermore, specific winemaking techniques can affect anthocyanin extraction and the residual content of anthocyanins in by-products [20]. Barbera by-products showed the highest anthocyanin content (1.82±0.09 mg/g cyanidin equivalents), which was from three to four times higher than the other cultivar by-products. These data are consistent with literature data that show that Barbera variety is very rich in these compounds, ranging between 4.00 to 12.00 mg/g of skin berry [21]. On this basis, Barberapomace can be considered a valuable source of anthocyanin compounds. However, when TAC of samples containing skins and seeds (*n*=5) was correlated with antioxidant activity, a positive but not significant correlation was observed (Table 4). The correlation between TPC, TAC and AOA was evaluated using Pearson's correlation coefficients (threshold for statistical significance: p<0.01).

Table 4. Correlation between parameters measured by spectrophotometric assays in the by-products containing anthocyanins (*n* = 5 samples).

Parameter	Pearson correlation coefficient (*r*)	statistical significance (p)	Strength
Total polyphenols vs Antioxidant capacity	*r* = 0.734	*P*<0.01	Positive strong correlation
Total anthocyanins vs Antioxidant capacity	*r* = 0.246	ns	Positive weak correlation
Total flavan-3-ols vs Antioxidant capacity	*r* = 0.520	*P*<0.05	Positive moderate correlation

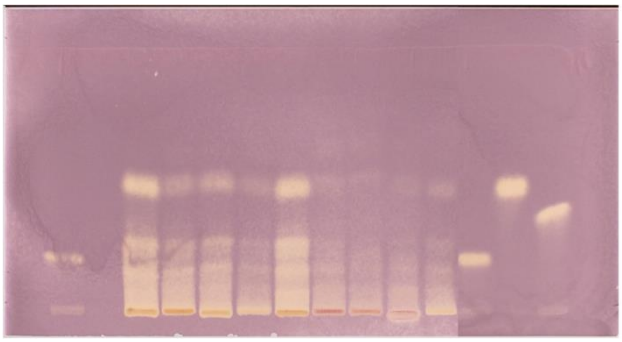
Data reported in Table 4 suggest that flavan-3-ols, compared to anthocyanins, contribute to a greater extent to the antioxidant activity.

3.2 Thin Layer Chromatography (HPTLC)

The HPTLC technique allowed a parallel evaluation of antioxidant activity and phenol compound distribution in grape samples.

3.2.1 Phenolic pattern and antioxidant activity of grape samples

The innovative approach of HPTLC technique allowed the correlation of polyphenol pattern with the relative antioxidant activity. For the evaluation of the antioxidant activity, the plates were exposed at 366 nm and visible light after derivatization with the DPPH solution. Figure 5 shows the phenol distribution and the associated antioxidant activity of samples included in this study (revealed at visible light after the derivatization step).



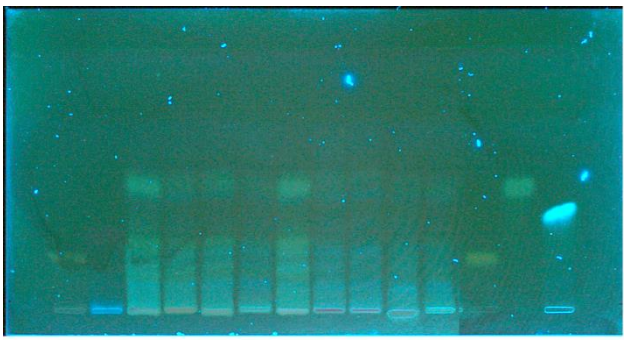
Q1 K S1 S2 S3 S4 S5 S6 S7 S8 S9 Hy EC CA

Figure 5. HPTLC patterns of samples after exposure of the plate at visible light and derivatization with DPPH solution. Standard flavonoids are run in parallel.

Q1 = Quercetin-3-O-glu
K = Kaempferol-3-O-glu
S1 = G-IF-S-15
S2 = G-AF-S-15
S7 = U-AF-SS-16
S8 = B-AF-SS-17
S9 = G-AF-SS-18
S3 = G-IF-S-16
S4 = G-AF-SS-16
S5 = U-IF-S-15
S6 = U-AF-SS-15
Hy = Hyperoside
EC = Epicatechin
CA = Caftaric acid

Generally speaking, after derivatization with DPPH solution and exposure of the plate at visible light, S1 (Grignolino seeds 2015 before fermentation), S2 (Grignolino seeds 2015 after fermentation), S3 (Grignolino seeds 2016 before fermentation) and S5 (Uvalino seeds 2015 before fermentation) were the samples with the highest polyphenol abundance and antioxidant capacity, as shown by the strong discoloration of the bands. In particular, a band corresponding to epicatechin (EC) was visible in all the samples included. This compound was very abundant in samples containing only seeds before the fermentation process: Grignolino 2015 and 2016, and Uvalino 2015. However, EC was also relatively abundant also in Grignolino 2015 at the end of fermentation. This is due to the high concentration of epicatechin in proanthocyanidins, the phenol compounds most abundant in the seeds. In addition, EC was also the compound with a high antioxidant capacity. These data are in agreement with spectrophotometric results (Table 2) where Grignolino samples were the most rich in polyphenols and proanthocyanidins, even after the fermentation process. Compared to the samples reported above, the other samples showed a lower content of polyphenols with antioxidant activity, probably due to the minor abundance of seeds and to the presence of skins, that generally show a very reduced content of polyphenols after the fermentation. In support of this hypothesis, Barbera sample (S8), that showed the highest content of anthocyanins, was characterized by a lower antioxidant activity.

Caftaric acid, quercetin-3-O-glucoside and hyperoside were not detectable in the sample analyzed, as shown in Figure 6, representing the phenol distribution of samples included in the study revealed at 366 nm after the derivatization step.



Q1 K S1 S2 S3 S4 S5 S6 S7 S8 S9 Hy EC CA

Figure 6. HPTLC patterns of samples after exposure of the plate at 366 nm and derivatization with DPPH solution. Standard flavonoids are run in parallel.

Q1 = Quercetin-3-O-glu
K = Kaempferol-3-O-glu
S1 = G-IF-S-15
S2 = G-AF-S-15
S7 = U-AF-SS-16
S8 = B-AF-SS-17
S9 = G-AF-SS-18
S3 = G-IF-S-16
S4 = G-AF-SS-16
S5 = U-IF-S-15
S6 = U-AF-SS-15
Hy = Hyperoside
EC = Epicatechin
CA = Caftaric acid

4 Conclusions

This study describes the characterization of the phenolic fraction of winemaking by-products using different analytical approaches. Spectrophotometric methods, although not specific, were useful to obtain preliminary information about phenolic composition of grape samples and were generally satisfactorily correlated with the antioxidant activity. In more detail, samples containing only seeds showed the highest total polyphenol content and the highest antioxidant capacity (R^2>0.9). The presence of the skins reduced the correlation among TPC and AOA (R^2=0.8), suggesting that proanthocyanidins are the main polyphenol compounds contributing to the antioxidant capacity. HPTLC confirmed the spectrophotometric data and showed interesting results not only for the screening of active compounds, but also for the evaluation of the antioxidant activity associated with each molecule (other *in vitro* antioxidant assays determine only the total activity). These preliminary results confirm that winemaking by-products contain, in different amount, phenolic substances with significant antioxidant activity, supporting their use as a source of healthy compound. In the literature, great attention is given to winery by-products, especially to grape skins [7, 19]. However, our data show that also the seeds can be recovered and used for different purposes, for example dietary supplement formulations, cosmetics or as preservatives in food products. Grape skins of same varieties show a very high content of anthocyanins after alcoholic fermentation. In this study, Barbera grape skins showed an unexpected content of anthocyanins; in our previous studies [22] Barbera variety was investigated for its *in vitro* anti-inflammatory properties at gastric level showing promising results.

On this basis, we can conclude that, even if with some differences, winemaking pomace could be successfully used for different healthy purposes, implementing, in parallel, the circular economy.

Finally, since this is a preliminary study aimed at a performing a first screening of phenol compounds, further studies will be conducted to identify and quantify the characterizing polyphenol compounds in the samples analysed.

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References

1. FAO-OIV, 2016. Focus on table and dried grapes Available at: https://www.oiv.int/public/medias/4911/fao-oiv-grapes-report-flyer.pdf
2. `OIV, 2022. State of the world vine and wine sector 2021 Available at: https://www.oiv.int/public/medias/8778/eng-state-of-the-world-vine-and-wine-sector-april-2022-v6.pdf
3. B. Jin, J.M. Kelly, Biotechnology for agro-industrial residues utilization. Wine Industry residues (Springer Science+Business Media, Berlin, 2009)
4. V. Novello, L' Informatore Agrario. **71**, 33 (2015)
5. M.A. Bustamante, R. Moral, C. Paredes, A. Perez-Espinosa, J. Moreno-Caselles, M.D. Perez-Murcia, Waste Management **28**, 372 (2008)
6. OIV, 2018. Managing by-products of vitivinicultural origin. Available at: https://www.oiv.int/public/medias/6268/managing-viticulture-by-products-print.pdf
7. A.K. Chakka, A.S. Babu, App. Food Res. **2**, 100058 (2022)
8. N.C Briggs, R.S. Levine, L.D. Bobo, W.P. Haliburton, E.A. Brann, C.H. Hennekens, Am. J. Epidemiol. **156**, 454 (2002)
9. S.C. Renaud, M. De Lorgeril, Lancet **339**, 1523 (1992)
10. T.T. Fung, K.M. Rexrode, C.S. Mantzoros, J.E. Manson, W.C. Willett, F.B. Hu, Circulation **119**, 1093 (2009)
11. V.L. Singleton, J.A. Rossi, Am. J. Enol. Viticult. **16**, 144 (1965)
12. AOAC Official Method 2005.02. 2005. J. AOAC Int. **88**, 1269 (2005)
13. L.G. Butler, M.L. Price, J.E. Brotherton, J. Agric. Food Chem. **30**, 1087 (1982)
14. W. Brand-Williams, M.E. Cuvelier, C. Berset, Food Sci. Technol. **28**, 25 (1995)
15. L.P. Leong, G. Shui. Food Chem. **76**, 69 (2002)
16. E. Reich, A. Schibli High Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants (CIC Edizioneinternazionale, 2006)
17. C. Rodriguez-Perez, B. Garcia-Villanova, E. Guerra-Hernandez, V. Verardo, Nutrients **11**, 2435 (2019)
18. D. Bagchi, A. Swaroop, H.G. Preuss, M. Bagchi, Mut. Res. **768**, 69 (2014)
19. N. Unisan, J. Funct. Foods **67**, 103861 (2020)
20. E. Costa, F. Cosme, A.M. Jordao, A. Mendes-Faia, Oeno-One **48**, 1 (2014)
21. M.A. Paissoni, S. Rio Segade, S. Giacosa, F. Torchio, F. Cravero, V. Englezos, K. Rantsiou, C. Carboni, V. Gerbi, P.L. Teissedre, L. Rolle, Food Res. Int. **98**, 68 (2017)
22. F. Colombo, C. Di Lorenzo, L. Regazzoni, M. Fumagalli, E. Sangiovanni, L. Peres de Sousa, L. Bavaresco, D. Tomasi, A. Bosso, G. Aldini, P. Restani, M. Dell' Agli, Food Funct. **10**, 1797 (2019)